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Review

Oxidation of the aromatic amino acids tryptophan and tyrosine disrupts their anabolic effects on bone marrow mesenchymal stem cells

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ABSTRACT

Age-induced bone loss is associated with greater bone resorption and decreased bone formation resulting in osteoporosis and osteoporosis-related fractures. The etiology of this age-induced bone loss is not clear but has been associated with increased generation of reactive oxygen species (ROS) from leaky mitochondria. ROS are known to oxidize/damage the surrounding proteins/amino acids/enzymes and thus impair their normal function. Among the amino acids, the aromatic amino acids are particularly prone to modification by oxidation. Since impaired osteoblastic differentiation from bone marrow mesenchymal stem cells (BMMSCs) plays a role in age-related bone loss, we wished to examine whether oxidized amino acids (in particular the aromatic amino acids) modulated BMMSC function. Using mouse BMMSCs, we examined the effects of the oxidized amino acids di-tyrosine and kynurenine on proliferation, differentiation and Mitogen-Activated Protein Kinase (MAPK) pathway. Our data demonstrate that amino acid oxides (in particular kynurenine) inhibited BMMSC proliferation, alkaline phosphatase expression and activity and the expression of osteogenic markers (Osteocalcin and Runx2). Taken together, our data are consistent with a potential pathogenic role for oxidized amino acids in age-induced bone loss.

Contents

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1. Introduction

Dietary modifications have been shown to impact the aging process. In particular caloric restriction in rodents of between 30 and 40% extends lifespan by over 30% (Mattson, 2005). Dietary protein restriction has also been shown to extend lifespan, and it has been proposed that the beneficial effect of caloric restriction is due in part to the decrease in dietary protein intake (Rizza et al., 2014; Solon-Biet et al., 2014). Depending on the model system examined, reductions in the sulfur-containing amino acids (in particular methionine), branched chain amino acids (leucine, isoleucine and valine) and serine, threonine and valine have all been associated with increase in longevity (Mirzaei et al., 2014). It is now well recognized that amino acids are signaling molecules in their own right and contribute to the regulation of whole body metabolism. Direct amino acid effects on islet B cells, hepatocytes and skeletal muscle cells appear to be mediated by calcium-binding receptors (Conigrave and Hampson, 2006). The calcium-sensing receptor (CaSR) is stereospecific for the L-isomer, indicating the existence of a specific amino acid binding site on the receptor (Conigrave et al., 2000).

Previous human studies that measured the post absorptive plasma concentrations of the large neutral amino acids in healthy subjects showed that Tryptophan (Trp) was the only amino acid to exhibit a significant response to age in males, consisting of a 14% decline in elderly subjects (Caballero et al., 1991). Trp is one of nine essential amino acids and plays a major role in several metabolic pathways including general protein synthesis, serotonin synthesis and kynurenine (Kyn) production (Jones et al., 2013). Kyn is an oxidation product for tryptophan and its levels are known to increase with aging and associated with a drop in tryptophan levels (Braidy et al., 2011). Elevated levels of Kyn pathway metabolites, such as quinolinic acid and others, have been observed (Beal et al., 1991) in several age-associated neuro-pathological conditions in rats as well as diseases involving immune activation in humans (Guillemin et al., 2005). Previous in vivo studies in young, middle-aged and old female Wistar rats showed that Trp levels and tryptophan 2, 3-dioxygenase (TDO) activity decreased in all tissues with age. Peak Trp concentrations were observed in the brain, liver and kidney of young adult rats (3 mo.) and subsequently decreased with advancing age. Brain Kyn content increased significantly with age while the Kyn content in the liver and kidney was significantly lower in middle-aged rats (12 mo.) than in young rats (3 mo.) but increased with advancing age to 24 months (Braidy et al., 2011).

Oxidative stress has been proposed to play a role in the aging process (Wang et al., 2014). The toxic effect of these free radicals can be ameliorated by cellular defense mechanisms such as antioxidants like ascorbic acid, glutathione and ubiquinol as well as reactive oxygen species (ROS)-detoxifying enzymes such as superoxide dismutase (SOD), which plays a role in eliminating O_2^- to produce H_2O_2 (Valentine et al., 2005). A cell is able to overcome small amounts of free radicals and maintain its original state; moderate oxidative stress can trigger apoptosis and high levels of ROS may cause necrosis (Valko et al., 2005). Age-related loss of bone mass and strength has been linked to increased ROS release from leaky mitochondria and their effects on bone cells (Manolagas, 2010). Indeed, studies have shown a decrease in osteoblast number and bone formation when mice were treated with a glutathione inhibitor (Jagger et al., 2005). Furthermore, studies using murine models of premature aging and signs of oxidative stress showed osteoporotic features (de Boer et al., 2002; Tyner et al., 2002). In vivo studies on mice showed an association between oxidative stress and a decrease in bone mineral density (BMD) (Basu et al., 2001; Maggio et al., 2003; Oh et al., 2007). Human clinical studies have also shown an effect of antioxidants on bone resorption (Pasco et al., 2006).

We have previously shown that aromatic amino acids (tyrosine, tryptophan and phenylalanine) activate distinct anabolic signaling pathways in BMMSCs (El Refaey et al., 2014). In the present study, we examined whether the effects of the aromatic amino acid oxides on BMMSCs were similar or different to those of the unmodified aromatic amino acid. We report that the oxidized amino acids (in particular Kyn) block BMMSC proliferation and osteogenic differentiation, and our findings suggest that age-related accumulation of these oxidized metabolites could play a pathogenic role in the decreases in bone mass seen with aging.

2. Material and methods

2.1. Isolation and culture of BMMSCs

All experiments were approved by the Institutional Animal Care and Use Committee at Georgia Regents University (Protocol #: BR09-11-265; Augusta, GA). The mice were housed in AAALAC-accredited facilities under the supervision of a veterinarian. Georgia Regents University complies with the NIH policy on animal welfare, the Animal Welfare Act, and all other applicable federal, state and local laws. Male C57BL/6 mice were purchased from the National Institute on Aging (Bethesda, MD, USA) aged rodent colony. BMMSCs were isolated from 18-month-old male C57BL/6 mice at the Georgia Regents University Stem Cell Core Facility as previously described (Zhang et al., 2008). In brief, six mice were euthanized by CO_2 overdose followed by thoracotomy. The femora and tibiae were dissected free of soft tissues and kept in cold phosphate-buffered saline (PBS) on ice. BMMSCs were isolated from the bone marrow of these long bones using a modified protocol (Peister et al., 2004; Tropel et al., 2004) by negative immune-depletion followed by positive immunoselection. Enriched BMMSCs were grown in Dulbecco's modified Eagle medium (cat#10-013; DMEM; Cellgro, Mediatech, Manassas, VA, USA) supplemented with 10% heat-inactivated fetal bovine serum (cat#S11150; FBS; Atlanta Biologicals, Lawrenceville, GA, USA) and